Association of the 276G→T polymorphism of the adiponectin gene with cardiovascular disease risk factors in nondiabetic Koreans[1–3]

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ABSTRACT

Background: The adiponectin gene is known to modulate adiponectin concentrations and diabetes mellitus development.

Objective: We assessed whether adiponectin gene variants contribute to circulating adiponectin, insulin resistance (IR), or cardiovascular disease risk factors.

Design: Nondiabetic subjects [$n = 902$; $\bar{x} \pm$ SE age: 42.5 ± 0.53 y; body mass index (BMI; in kg/m^2): 24.7 ± 0.11] were genotyped for 2 single-nucleotide polymorphisms (SNPs), 45T→G and 276G→T.

Results: After adjustment for age, sex, and BMI, subjects with the *G* allele for the SNP 276 had significantly higher concentrations of triacylglycerol and small dense LDL (sdLDL) and smaller LDL particle size than did *T/T* subjects. *G/G* subjects at SNP 276 had significantly lower plasma adiponectin and higher homeostasis model assessment (HOMA) of IR and urinary prostaglandin F2α than did *T/T* subjects. In the SNP 45-276 haplotype test, we also observed that subjects with the *X/X* haplotype had significantly higher plasma adiponectin after adjustment than did *TG/TG* or *TG/X* haplotype subjects. In the highest BMI group (BMI ≥ 26), *T/T* subjects had lower HOMA-IR ($P = 0.011$) and higher plasma adiponectin ($P = 0.026$) at SNP 276 than did *G/G* or *G/T* subjects. These patterns were also seen for adiponectin in haplotype groups. However, no significant genotype effect for SNP 45T→G was observed.

Conclusions: The 276G→T polymorphism of the adiponectin gene modulates circulating adiponectin and IR, particularly in obese states. *G* allele carriers also have higher oxidative stress, higher sdLDL concentrations, and smaller LDL particle size. Therefore, the presence of the *G* allele in the adiponectin gene at SNP 276 could be a significant contributor to higher cardiovascular disease risk in Koreans, independent of common environmental factors. *Am J Clin Nutr* 2005;82:760–7.

KEY WORDS Adiponectin, 267G→T, obesity, insulin resistance, cardiovascular disease risk

INTRODUCTION

Circulating concentrations of adiponectin, an adipocyte-derived protein, correlate inversely with severity of insulin resistance (IR) (1, 2) and with obesity, type 2 diabetes, and cardiovascular disease (CVD), all of which are closely related to IR (3–6). Genetic variability in adiponectin has been inconsistently associated with low serum adiponectin, IR, diabetes, and CVD (7, 8).

The adipocyte-, C1Q-, and collagen domain–containing (*ACDC*) gene consists of 3 exons and 2 introns and is located on chromosome 3q27, in the same region where a susceptibility locus for type 2 diabetes and measures of adiposity have been mapped (9, 10). Two *ACDC* single-nucleotide polymorphisms (SNPs)—ie, the 45T→G in exon 2 and the 276G→T in intron 2—were shown to be associated with type 2 diabetes in a Japanese population (8). In diabetic whites, the 276G→T polymorphism was observed to be a predictor of coronary artery disease (CAD) risk (11). In addition, the SNP 45T→G, either independently (12) or as a haplotype together with SNP 276G→T (7), was strongly associated with obesity and IR syndrome in nondiabetic German and Italian whites. However, these associations were not replicated by Swedish (13) and French (14) investigators. Therefore, the findings associated with the *ACDC* locus may present considerable heterogeneity among populations (15).

Our primary goal was to examine the association between the 2 *ACDC* polymorphisms and concentrations of circulating adiponectin and IR in a group of healthy Koreans who genetically and environmentally differ from previously reported studies in whites. In addition, we examined associations with other, novel CVD risk factors including visceral fat accumulation, small LDL particle size, lipid peroxides, and C-reactive protein (CRP), which may be closely related to low circulating concentrations of adiponectin and IR.

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SUBJECTS AND METHODS

Study subjects

Nine hundred two nondiabetic subjects were recruited consecutively from among ≈2500 participants in a prospective human genetic study that is supported by a Genome Research Development Project on Health and Medicine from the Korean Ministry of Health and Welfare (project no. 00-PJ3-PG6-GN-01-0001). None of the subjects were taking any medication or had a diagnosis of coronary vascular disease, diabetes mellitus, or cancer. Diabetes was ascertained according to the American Diabetes Association criteria (17) in which diabetes is defined as a fasting plasma glucose concentration ≥7 mmol/L or current treatment with antidiabetic agents.

Written informed consent was obtained from all subjects. The protocol was approved by the Institutional Review Board of Yonsei University.

Blood collection

Venous blood specimens were collected in EDTA-treated and plain tubes after a 12-h fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory room (within 1–3 h) and stored at −70 °C until analysis.

Genotyping

Genomic DNA was extracted from 5 mL whole blood by using a commercially available DNA isolation kit (WIZARD Genomic DNA purification kit; Promega Corp, Madison, WI) according to the manufacturer's protocol. We first screened 7 sites of previously reported *ACDC* SNPs (−11377C→G at proximal promoter, 45T→G at exon 2, 276G→T at intron 2, and H241P, Y111H, G90S, and R221S at exon 3) in 48 subjects (ratio of males to females, 1:1; ratio of normal-weight subjects to overweight or obese subjects, 1:1) to identify the allele frequency of each SNP. Each genotyping was performed with assays using single-primer extension technology (SNP-IT, SNPstream 25K System; Orchid Biosystems, Princeton, NJ). The DNA fragments were visualized by using ultraviolet illumination and an image analyzer (AlphaImager 1220; Alpha Innotech Corp, San Leandro, CA). The pUC19 DNA/*Msp* I (*Hpa* II) marker (MBI Fermentas, Vilnius, Lithuania) served as a control standard.

Anthropometric and blood pressure measurements

Body weight and height were measured in the morning while the subjects were unclothed and not wearing shoes. Body mass index (BMI) was calculated as body weight (in kg) divided by height (in m^2). Circumferences of waist and hip were measured while the subjects were standing after normal expiration, and the waist-to-hip ratio was also computed. Blood pressure was read from the left arm by using automatic blood pressure monitor (TM-2654; A&D, Tokyo, Japan) while subjects remained seated after a 20-min rest. An average of 3 measurements was recorded for each subject.

Assessment of food intake and physical activity level

Information about habitual food intake was obtained by using a 24-h recall method and a semiquantitative food-frequency questionnaire. We used the former to carry out analyses and the latter to ascertain whether the data collected by 24-h recall methods were representative of the usual dietary pattern. Nutrient intake data were calculated as mean values from a 3-d food record (2 weekdays and 1 weekend) through the 24-h recall method by using the database of the computerized Korean food code, based on food composition tables developed by National Rural Living Science Institute in Korea (17). Total energy expenditure (kcal/d) was calculated from activity patterns including basal metabolic rate, physical activity for 24 h (18), and specific dynamic action of food. The basal metabolic rate for each subject was calculated by using the Harris-Benedict equation (19).

Abdominal fat distribution at the first and fourth lumbar vertebrae as measured with a computerized tomographic scanner

Abdominal fat areas were measured by using computerized tomography scanning with a General Electric (GE) High-Speed Advantage 9800 scanner (GE, Milwaukee, WI). Two cross-sectional images were made for each subject: the abdomen at the levels of the first (L1) and fourth (L4) lumbar vertebra. Each computed tomographic slice was analyzed for the cross-sectional area of fat by using a density-control program available in the standard GE computer software. Parameters for total abdominal fat density at L1 and L4 were selected between the range of −150 and −50 Hounsfield units. Total abdominal fat area was divided into visceral and subcutaneous fat areas to calculate specific fat areas.

Serum lipid profile

Fasting serum concentrations of total cholesterol and triacylglycerol were measured by using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd, Tokyo, Japan). After precipitation of serum chylomicron, LDL, and VLDL by using dextran sulfate-magnesium, HDL cholesterol left in the supernatant was measured by an enzymatic method. LDL cholesterol was estimated indirectly by using the formula of Friedewald et al (20) for subjects with serum triacylglycerol concentrations <4.52 mol/L (400 mg/mL) and directly measured for subjects with serum triacylglycerol concentrations ≥4.52 mol/L. Serum apolipoprotein A-I and B were determined by turbidometry at 340 nm using a specific antiserum (Roche, Basel, Switzerland).

LDL subfraction and LDL particle size

The LDL subfraction was reported previously (21). According to the method described by Griffin et al (22), total LDL (density: 1.019–1.063 g/mL) was isolated by sequential density gradient ultracentrifugation. Three LDL subfractions (LDL_1: 1.025–1.034; LDL_2: d1.034–1.044; and LDL_3: d1.044–1.060 g/mL density) were quantified in fresh plasma by using nonequilibrium density ultracentrifugation. The percentage areas under the curve for each individual subfraction were quantified (Data Graphics; Beckman, High Wycombe, United Kingdom) and corrected for differential absorption characteristics of total lipoproteins by using extinction coefficients: 2.63 for LDL_1, 2.94 for LDL_2, and 1.96 for LDL_3. This coefficient was calculated as a percentage of total LDL (d1.019–1.063 g/mL) mass and was expressed in milligrams of lipoprotein per 100 mL plasma. Particle size distribution of LDL (density: 1.019–1.063 g/mL) isolated by sequential floatation ultracentrifugation was examined by using a pore- gradient lipoprotein system (CBS Scientific, Del Mar, CA) with commercially available, nondenaturing polyacrylamide slab gels containing a linear gradient of 2–16% acrylamide (Alamo Gels Inc, San Antonio, TX). Standards

of latex beads (34 nm), thyroglobulin (17 nm), apoferritin (12.2 nm), and catalase (10.4 nm) were used to estimate the relative migration rates of each band. The gels were scanned by using a GS-800 Calibrated Imaging Densitometer (Bio-Rad Laboratories, Graz, Austria). LDL particle size was calculated with reference to the relative migration value of the standards.

Glucose, insulin, free fatty acids, and homeostasis model assessment of insulin resistance

Fasting glucose was measured by using a glucose oxidase method and a glucose analyzer (Beckman Instruments, Irvine, CA). Insulin was measured by using radioimmunoassays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN). Free fatty acids (FFAs) were analyzed by using a Hitachi 7150 autoanalyzer (Hitachi Ltd, Tokyo, Japan). IR was calculated by using HOMA according to the following equation:

$$\text{IR} = [\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose (mmol/L)}]/22.5 \quad (1)$$

Lipid peroxidation

After a 12-h fast and before blood collection, urine was colleted into polyethylene bottles containing 1% butylated hydroxytoluene. The tubes were immediately covered with aluminum foil and stored at −70 °C until analysis. We measured 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) by using an enzyme immunoassay (BIOXYTECH Urinary 8-epi-$PGF_{2\alpha}$ Assay kit; OXIS International Inc, Portland, OR). The resulting color reaction was read using a Victor^2 multilabel counter (Perkin Elmer Life Sciences, Turku, Finland) at 650 nm. Urinary creatinine was measured by the alkaline picrated (Jaffe) reaction (23), and urinary 8-epi-$PGF_{2\alpha}$ concentrations were expressed as pmol/mmol creatinine. Plasma malondialdehyde was assayed according to the fluorometric method of Buckingham (24).

Plasma concentration of C-reactive protein and adiponectin

Plasma CRP was measured by isomg an Express Plus autoanalyzer (Chiron Diagnostics Co, Walpole, MA) and a commercially available high-sensitivity kit [CRP-Latex (II) X2; Seiken Laboratories Ltd, Tokyo, Japan] (25). Plasma adiponectin concentrations were measured by using an enzyme immunoassay (Human Adiponectin ELISA kit; B-Bridge International Inc, Sunnyvale, CA). The resultant color reaction was read at 450 nm by using a Victor^2 multilabel counter.

Statistical analysis

We used SPSS for WINDOWS software (version 11.0; SPSS Inc, Chicago, IL) for all statistical analyses. Of the screened *ACDC* SNPs, we excluded SNPs with missense mutations of allele frequency $< 2\%$ and included the others in the analysis. To examine whether each of the selected SNPs was in Hardy-Weinberg equilibrium and whether the SNPs were in linkage disequilibrium, we performed an analysis by using the Executive SNP Analyzer, version 1.0 (*see* http://www.istech.info/SilicoSNP/index.html?). To evaluate the correlation of the variables, we used Pearson's correlation coefficient. We evaluate the influence of selected SNPs on continuous variables by using a general linear model and then the Bonferroni test to adjust for covariates such as age, sex, and BMI, which were highly correlated to IR, and adiponectin concentration. Each variable was examined for normal distribution, and significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented on untransformed and unadjusted variables. Results are expressed as means ± SEs. A 2-tailed value of $P < 0.05$ was considered significant.

RESULTS

Detection of SNPs in the *ACDC* gene

Among the 7 screened polymorphisms in the *ACDC* gene, we found 5 rare, nonsynonymous mutations (−11391G→A at promoter region and H241P, Y111H, G90S, and R221S at exon 3; allele frequency $< 2\%$) in healthy Koreans. The allele frequencies of these SNPs in the *ACDC* gene were as follows: −11391G→A (G:A = 1:0), H241P (A:C = 1:0), Y111H (T:C = 1:0), G90S(G:A = 1:0), and R221S (C:A = 0.98:0.02). Therefore, we included SNPs 45T/G and 276G/T in the *ACDC* gene to further analysis.

Distribution of 45T/G, 276 G/T, and 45/+276 haplotypes of the *ACDC* gene

Genotype distributions were in Hardy-Weinberg equilibrium: 49.1% of *T/T*, 42.8% of *T/G*, and 8.1% of *G/G* was at SNP 45, and 50.0% of *G/G*, 41.7% of *G/T*, and 8.3% of *T/T* was at SNP 276. *T* is the major allele at position 45 (*T* frequency = 0.705; $P = 0.377$), and *G* is the major allele at position 276 (*G* frequency = 0.708; $P = 0.784$); these frequencies are not significantly different from those reported in nondiabetic Japanese (*T* frequency at position 45 = 0.714; *G* frequency at position 276 = 0.703) (8). These 2 polymorphisms were in linkage disequilibrium ($|'D| = 1$; $P = 0.0008$), and there were estimated 45/276 haplotype frequencies of 17.0% for *TG/TG*, 23.8% for *TG/TT*, 24.9% for *TG/GG*, 8.3% for *TT/TT*, 17.8% for *TT/GG*, and 8.1% for *GG/GG*. For subsequent statistical analyses, subjects were divided into 3 genotype subgroups: homozygous for the TG haplotype (*TG/TG*; $n = 153$), heterozygous carriers of the TG haplotype (*TG/X*; $n = 440$) and non-TG haplotype carriers (*X/X*; $n = 309$).

Clinical characteristics and body fat distribution according to genotype

The clinical characteristics, abdominal fat area, and blood pressure of healthy subjects according to the *45T→G* and *276G→T* genotypes are shown in **Table 1**. There were no significant genotype-related differences in age, sex distribution, BMI, waist-to-hip ratio, percentage total body fat, visceral and subcutaneous fat areas at L1 and L4, or blood pressure. Likewise, there were no differences among genotypes for cigarette smoking, alcohol consumption, energy intake, or TEE (**Table 2**). For the 45/276 haplotype, we could not observe any significant association with clinical characteristics and body fat distribution (data not shown).

Serum lipid profiles and small dense LDL according to genotype

There were no significant genotype-related differences among SNP *45T→G* and *276G→T* genotypes with respect to serum concentrations of HDL, LDL, and total cholesterol and apolipoproteins A-I and B (**Table 3**). Serum triacylglycerol concentrations and small dense LDL (LDL_3) concentrations (both: **Figure 1**) were significantly higher in subjects with the *G/G* and *G/T*

TABLE 1
Age, body fat distribution, and blood pressure according to the genotypes of the ACDC *45T→G* and *276G→T* genotypes in healthy Koreans[1]

	SNP 45T→G			SNP 276G→T		
	T/T (n = 443)	*T/G* (n = 386)	*G/G* (n = 73)	*G/G* (n = 451)	*G/T* (n = 376)	*T/T* (n = 75)
Age (y)	43 ± 1[2]	42 ± 0.83	43 ± 2	43 ± 1	42 ± 1	44 ± 2
Sex (M/F)	156/287	146/240	29/44	168/283	141/235	22/53
BMI (kg/m^2)	24.8 ± 0.16	24.7 ± 0.18	24.5 ± 0.37	24.7 ± 0.16	24.6 ± 0.18	24.9 ± 0.40
Waist-hip ratio	0.87 ± 0.00	0.87 ± 0.00	0.88 ± 0.01	0.88 ± 0.00	0.87 ± 0.00	0.87 ± 0.01
Fat (%)	29.6 ± 0.40	29.6 ± 0.44	28.3 ± 0.92	29.6 ± 0.41	29.3 ± 0.43	30.6 ± 0.91
First lumbar vertebra						
Visceral fat (cm^2)	92.1 ± 2.78	92.1 ± 3.32	97.2 ± 6.02	93.2 ± 2.86	90.9 ± 3.09	96.6 ± 7.36
Subcutaneous fat (cm^2)	130.6 ± 3.54	136.6 ± 4.41	123.1 ± 7.59	131.0 ± 3.62	132.5 ± 4.16	141.1 ± 9.35
Fourth lumbar vertebra						
Visceral fat (cm^2)	79.4 ± 2.11	77.6 ± 2.57	80.4 ± 4.45	80.0 ± 2.22	76.3 ± 2.22	84.2 ± 6.40
Subcutaneous fat (cm^2)	188.2 ± 4.24	195.2 ± 4.82	182.1 ± 7.28	188.8 ± 4.11	190.8 ± 4.78	200.2 ± 10.1
Blood pressure (mm Hg)						
Systolic	122 ± 1	122 ± 1	119 ± 2	121 ± 1	122 ± 1	122 ± 2
Diastolic	75 ± 1	76 ± 1	76 ± 1	76 ± 1	76 ± 1	75 ± 1

[1] SNP, single-nucleotide polymorphism. There were no significant differences between the *45T→G* and *276G→T* genotypes on the basis of one-way ANOVA or one-way analysis of covariance by using a general linear model followed by Bonferroni test after adjustment for covariates such as age, sex, and BMI.
[2] $\bar{x}$ ± SE (all such values).

genotypes of SNP 276G→T than in those with *T/T* after adjustment for possible confounding effects of age, sex, and BMI. Moreover, LDL_2 percentage and LDL particle size were significantly lower in G carriers of SNP 276G→T than in those with *T/T* (Table 3). However, there were no significant genotype effects of SNP 45 on triacylglycerol, small dense LDL concentrations, or LDL particle size. In addition, mean values of these variables did not differ significantly between the 45/276 haplotype groups (data not shown).

Homeostasis model assessment of insulin resistance and circulating adiponectin, C-reactive protein, and urinary prostaglandin $F_{2\alpha}$ concentrations according to genotype

Subjects carrying G/G homozygotes at position 276 had significantly lower concentrations of plasma adiponectin and higher HOMA-IR and urinary excretion of $PGF_{2\alpha}$ than did those with T/T after adjustment for possible confounding effects of age, sex, and BMI (**Table 4**). However, there were no significant genotype effects of SNP 45T→G on circulating adiponectin concentrations, HOMA-IR, plasma CRP, or urinary excretion of $PGF_{2\alpha}$. Serum insulin concentrations tended to be higher in the subjects with the *GG* genotype for 276G→T, but the difference was not significant. In addition, serum glucose and plasma CRP concentrations did not differ significantly between genotype groups at SNPs 45 and 276. In the 45/276 haplotype test, subjects with X/X haplotype had significantly higher concentrations of plasma adiponectin after adjustment for possible confounding effects of age, sex, and BMI (TG/TG: 6.11 ± 0.30 μg/mL; TG/X: 6.06 ± 0.18 μg/mL; X/X: 7.16 ± 0.24 μg/mL; $P = 0.007$). However, there were no significant haplotype effects of the 45/276 haplotype on HOMA-IR, plasma CRP, and urinary excretion of $PGF_{2\alpha}$ (data not shown).

Relation of adiponectin, triacylglycerol concentrations, and homeostasis model assessment of insulin resistance to LDL particle size

Pearson correlation test showed that LDL particle size had highly significant negative relations with serum fasting triacylglycerol concentrations ($r = -0.450$, $P < 0.001$), HOMA-IR ($r = -0.215$, $P < 0.001$), and LDL_3 ($r = -0.513$, $P < 0.001$) in

TABLE 2
Cigarette smoking, alcohol consumption, energy intake, and total energy expenditure according to the ACDC *45T→G* and *276G→T* genotypes in healthy Koreans[1]

	SNP 45T→G			SNP 276G→T		
	T/T (n = 443)	*T/G* (n = 386)	*G/G* (n = 73)	*G/G* (n = 451)	*G/T* (n = 376)	*T/T* (n = 75)
Smoking (cigarettes/d)	15.0 ± 0.71	14.1 ± 0.80	14.9 ± 2.48	16.3 ± 0.79	12.9 ± 0.80	13.8 ± 1.46
Alcohol (g/d)	17.1 ± 1.88	16.6 ± 2.27	22.3 ± 8.44	18.3 ± 2.37	17.5 ± 2.16	15.1 ± 2.23
TEI (kcal/d)	2328 ± 20	2332 ± 23	2345 ± 49	2322 ± 21	2345 ± 22	2315 ± 48
TEE (kcal/d)	2310 ± 19	2341 ± 21	2288 ± 49	2323 ± 19	2322 ± 21	2300 ± 50
TEI:TEE	1.04 ± 0.01	1.03 ± 0.01	1.03 ± 0.01	1.03 ± 0.01	1.04 ± 0.01	1.04 ± 0.01

[1] All values are $\bar{x}$ ± SE. SNP, single-nucleotide polymorphism; TEI, total energy intake; TEE, total energy expenditure. There were no significant differences between the *45T→G* and *276G→T* genotypes on the basis of one-way analysis of covariance by using a general linear model followed by Bonferroni test after adjustment for covariates such as age, sex, and BMI.

TABLE 3
Serum lipid profiles, LDL subfractions, and LDL particle size according to the *ACDC 45T→G* and *276G→T* genotypes in healthy Koreans[1]

	SNP 45T→G			SNP 276G→T		
	T/T (n = 443)	*T/G* (n = 386)	*G/G* (n = 73)	*G/G* (n = 451)	*G/T* (n = 376)	*T/T* (n = 75)
Total cholesterol (mg/dL)	198.4 ± 1.74	200.6 ± 1.96	197.9 ± 4.58	200.7 ± 1.71	198.9 ± 2.04	193.4 ± 4.21
LDL cholesterol (mg/dL)	124.9 ± 1.58	126.1 ± 1.75	122.4 ± 4.01	125.7 ± 1.54	125.1 ± 1.80	122.9 ± 4.21
HDL cholesterol (mg/dL)	47.3 ± 0.58	47.1 ± 0.59	46.6 ± 1.27	46.7 ± 0.51	47.6 ± 0.61	48.0 ± 1.82
Apo A1 (mg/dL)	138.7 ± 1.16	139.4 ± 1.34	140.7 ± 2.62	139.2 ± 1.16	139.4 ± 1.29	138.0 ± 3.13
Apo B (mg/dL)	87.3 ± 1.28	87.8 ± 1.39	85.3 ± 3.17	87.7 ± 1.25	87.3 ± 1.46	85.8 ± 2.91
LDL_1 (%)	17.1 ± 1.21	15.4 ± 1.64	13.6 ± 7.9	14.2 ± 1.53	16.8 ± 1.34	20.8 ± 3.10
LDL_2 (%)	55.3 ± 2.03	50.6 ± 3.28	47.5 ± 8.56	50.9 ± 3.04^b	51.6 ± 2.32^b	65.4 ± 2.93^a
LDL_3 (%)	27.6 ± 2.37	34.0 ± 4.03	39.0 ± 11.1	34.9 ± 3.57^a	31.6 ± 2.97^a	14.0 ± 1.11^b
LDL particle size (nm)	25.6 ± 0.05	25.7 ± 0.06	25.7 ± 0.13	25.6 ± 0.05^b	25.6 ± 0.06^b	25.9 ± 0.11^a

[1] All values are $\bar{x}$ ± SE. SNP, single-nucleotide polymorphism; Apo, apolipoprotein. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (one-way analysis of covariance by using a general linear model followed by Bonferroni test after adjustment for covariates such as age, sex, and BMI).

healthy subjects. Moreover, significant correlations were observed between plasma adiponectin and LDL particle size (r = 0.202, $P < 0.001$) and serum triacylglycerol concentrations (r = −0.308, $P < 0.001$).

Relation of serum insulin and homeostasis model assessment of insulin resistance to oxidative stress

Urinary excretion of $PGF_{2\alpha}$ showed a highly significant positive relation with serum insulin (r = 0.257, $P < 0.001$) and HOMA-IR (r = 0.239, $P < 0.001$) in healthy subjects. However, we did not find a significant correlation between plasma adiponectin concentrations and the urinary excretion of $PGF_{2\alpha}$.

Relation between SNP 276G→T and adiponectin concentrations and homeostasis model assessment of insulin resistance according to BMI tertiles

We found a significantly positive correlation between HOMA-IR and BMI (r = 0.462, $P < 0.001$) and significant negative correlations between plasma adiponectin concentration and HOMA-IR (r = −0.248, $P < 0.001$) and BMI (r = −0.248, $P < 0.001$). In addition, we observed an interaction between BMI and genotype at position 276 for HOMA-IR ($P < 0.001$) and plasma adiponectin concentration ($P < 0.001$). Therefore, we performed a subset analysis by dividing the subjects into subgroups according to BMI tertiles. As shown in **Figure 2**, there were no significant differences in HOMA-IR and plasma adiponectin concentrations between the *SNP 276G→T* genotypes in the lean (BMI ≤ 23.2) and the intermediate (23.2 < BMI < 26.0) subgroups. However, in the obese subgroup (BMI ≥ 26.0), we observed significant differences in HOMA-IR (G/G: 2.68 ± 0.11; G/T: 2.60 ± 0.19; T/T: 1.89 ± 0.15; P = 0.011) and adiponectin concentrations (G/G: 5.39 ± 0.25; G/T: 5.74 ± 0.30; T/T: 7.08 ± 0.62 μg/mL; P = 0.026) according to the *SNP 276G→T* genotypes. We also found a significant ($P < 0.001$) interaction between BMI and the 45/276 haplotype for plasma adiponectin concentrations. Significant differences in adiponectin concentrations (TG/TG: 6.11 ± 0.30; G/T: 6.06 ± 0.18; T/T: 7.16 ± 0.24 μg/mL; P = 0.020) were observed only in the subjects in the highest BMI tertile. However, the *ACDC 45T→G* genotypes were not associated with HOMA-IR and plasma adiponectin concentrations according to the BMI tertiles (data not shown).

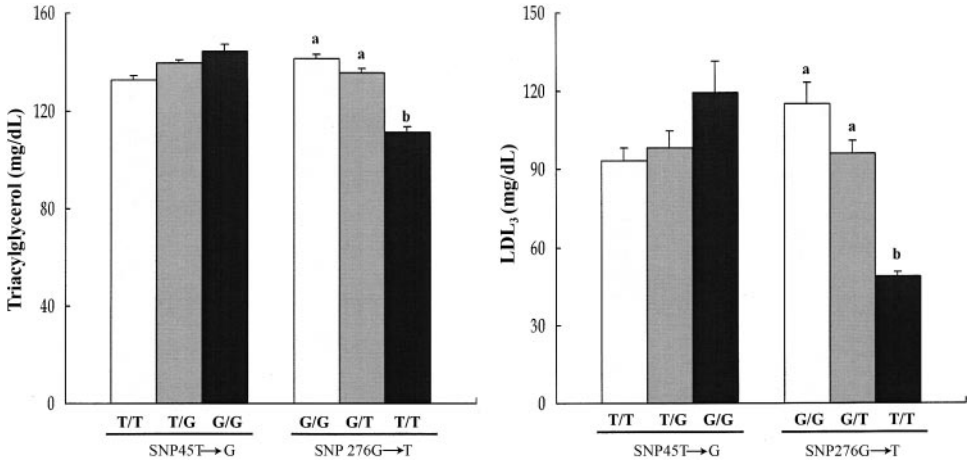

FIGURE 1. Serum triacylglycerol and small dense LDL (LDL_3) concentrations according to *ACDC* 45T→G and 276G→T in healthy subjects. Means with different superscript letters in SNP 45 or 276 are significantly different, $P < 0.05$ (one-way analysis of covariance with a general linear model followed by Bonferroni test after adjustment for covariates such as age, sex, and BMI).

TABLE 4
Circulating glucose, insulin, adiponectin, and C-reactive protein (CRP) concentrations and urinary prostaglandin F2α ($PGF_{2\alpha}$) concentrations according to the *ACDC 45T→G* and *276G→T* genotypes in healthy Koreans[1]

	SNP 45T→G			SNP 276G→T		
	T/T (n = 443)	*T/G* (n = 386)	*G/G* (n = 73)	*G/G* (n = 451)	*G/T* (n = 376)	*T/T* (n = 75)
Glucose (mg/dL)	84.4 ± 0.52	85.9 ± 0.63	85.2 ± 1.28	85.3 ± 0.53	85.2 ± 0.63	83.4 ± 1.24
Insulin (μIU/mL)	9.19 ± 0.31	9.67 ± 0.37	8.43 ± 0.45	9.49 ± 0.29	9.45 ± 0.40	7.91 ± 0.42
HOMA-IR	1.92 ± 0.07	2.06 ± 0.08	1.77 ± 0.10	2.01 ± 0.06[a]	1.98 ± 0.08[a,b]	1.65 ± 0.10[b]
Adiponectin (μg/mL)	6.40 ± 0.18	6.41 ± 0.22	6.89 ± 0.39	6.22 ± 0.17[b]	6.43 ± 0.22[a,b]	7.67 ± 0.41[a]
CRP (mg/dL)	0.15 ± 0.01	0.19 ± 0.02	0.18 ± 0.03	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.03
$PGF_{2\alpha}$ (pg/mg creatinine)	1030.9 ± 44.1	1080.3 ± 42.0	1243.6 ± 129.5	1123.1 ± 46.4[a]	1054.6 ± 44.1[a,b]	838.1 ± 61.8[b]

[1] All values are $\bar{x}$ ± SE. HOMA-IR, homeostasis model assessment of insulin resistance, calculated as [fasting insulin (μIU/mL) × fasting glucose (mmol/L)]/22.5. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (one-way analysis of covariance by using a general linear model followed by Bonferroni test after adjustment for covariates such as age, sex, and BMI).

DISCUSSION

The current study shows a significant association between *ACDC 276G→T* genotypes and adiponectin concentrations and HOMA-IR in healthy Korean subjects after adjustment for potential confounders, including age, sex, and BMI. These findings are consistent with a previous report (8) showing that *ACDC 276G→T* genotypes were associated with IR, which could be mediated through alterations on the expression level that subsequently affected plasma adiponectin concentrations. It is interesting that we observed in the current study that these effects were significant only in the population-specific highest tertile of BMI (BMI ≥26).

The current results suggest that the genotype effects of the adiponectin gene on circulating adiponectin and IR are more significant in overweight or obese subjects than in lean subjects. Similarly, Hara et al (8) found that the *G* allele at position 276 was linearly associated with lower plasma adiponectin concentrations only in subjects in the highest BMI tertile (BMI ≥ 26.7). It should be noted that this observation within the *ACDC* locus, by which the phenotypic expression of the genotype is observed primarily among subjects with elevated BMI or other measures of obesity. is consistent with observations from many other CVD candidate genes (26, 27). Reports have shown that low circulating adiponectin concentrations predicted the risk of developing IR or type 2 diabetes in small Pima Indian cohorts (28, 29) and in a larger and genetically heterogeneous white cohort (30, 31). It has been suggested that IR is a consequence rather than a predictor of decreased adiponectin expression in adipose tissue (8).

Urinary excretion of $PGF_{2\alpha}$, a sensitive marker of oxidative stress (32), was observed to be high under conditions that predispose to CAD, such as IR, hypertension, and hypercholesterolemia (33, 34). In fact, most CAD risk factors, including those involved in the metabolic syndrome, are known to be closely associated with IR. Therefore, the association between the *ACDC* 276G→T polymorphism and IR might provide the basis for the observed association between the presence of the G allele and higher urinary excretion of $PGF_{2\alpha}$.

An important and novel contribution from this study relates to the identified association between the *ACDC* 276G→T polymorphism and small dense LDL (LDL_3) and LDL particle size, both of which are considered CAD risk factors (35). In the current study, carriers of the G allele at *ACDC* 276G→T had significantly higher mean LDL_3 concentrations and smaller LDL particle size than did subjects with the *T/T* genotype, which potentially places the former group of subjects at a significantly higher risk of CAD (22).

High LDL_3 concentrations have been associated with visceral fat accumulation (36) and a carbohydrate-rich diet in Koreans (37). However, in the current study, we did not observe significant differences in abdominal body fat areas and usual carbohydrate intakes between genotype groups for SNP 276G→T. A strong independent association between small LDL particle size

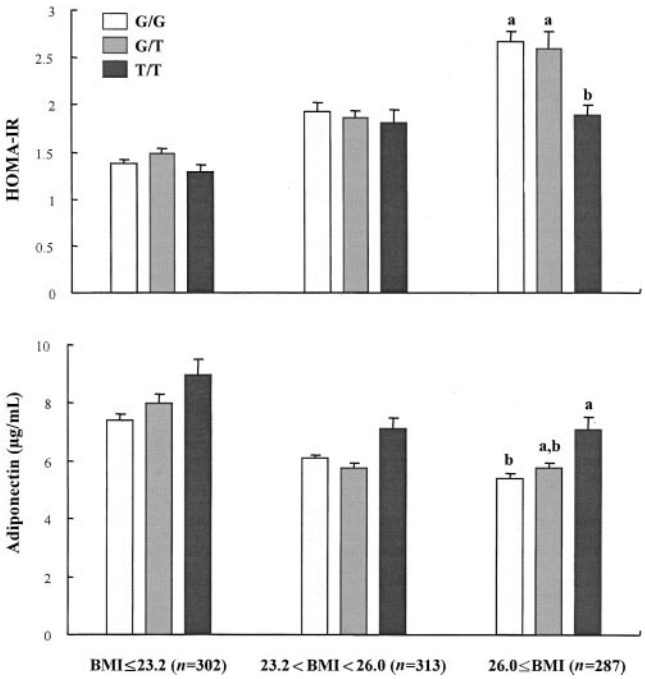

FIGURE 2. Effects of the *SNP 276* genotype on homeostasis model assessment of insulin resistance [HOMA-IR = [fasting insulin (μIU/mL) × fasting glucose (mmol/L)]/22.5] and plasma adiponectin concentrations in healthy subjects. Means with different superscript letters in SNP 45 or 276 are significantly different, $P < 0.05$ (analysis of covariance with a general linear model followed by Bonferroni test after adjustment for covariates such as age, sex, and BMI).

and low adiponectin concentrations was observed (36, 38). In addition, LDL_3 concentrations and small LDL particle size were reported to be associated with high fasting triacylglycerol concentrations (21) and high IR (39). Therefore, the high concentrations of LDL_3 and small LDL particle size in *G* carriers might be related to the high IR and triacylglycerol concentrations and low circulating adiponectin that are associated with the genetic variation at the adiponectin locus.

In the SNP 45 and 276 haplotype test, we observed that, compared with TG/TG or TG/X haplotype groups, subjects with the X/X haplotype (non-TG haplotype carriers) had significantly higher concentrations of plasma adiponectin after adjustment for age, sex and BMI, but there were no significant haplotype effects on HOMA-IR. In addition, we found that carriers of the X/X haplotype in the highest BMI tertile group had significantly higher adiponectin concentrations, which were similar to those shown in SNP 276G→T. However, we did not observe any significant genotype-related associations between *ACDC 45T→G* genotypes and circulating adiponectin and IR concentrations.

This finding is similar to the findings of previous studies by Bacci et al (11). According to that report, SNP 276 was strongly associated with CAD risk and IR, and the association was also shown in the haplotype of SNPs 45 and 276. However, SNP 45 alone did not have any significant association. Menzaghi et al (7) also found a similar association between both SNPs and HOMA-IR in nondiabetic white subjects, but the strongest association was with a haplotype defined by both *ACDC* 45T→G and 276G→T. Considering these results, we assumed that the association with the 45/276 haplotype is due to the fact that SNP 45 is in strong linkage disequilibrium with SNP276. Therefore, SNP 276 polymorphism might be a better predictor of CVD risk than is SNP 45 polymorphism. On the other hand, *ACDC* 45T→G was significantly associated with obesity and insulin sensitivity in a German population without a family history of diabetes (12). Possible explanations for this discrepancy include differences in family history status and anthropometric and ethnic factors.

In summary, the association of SNP 276 genotypes of the adiponectin gene with circulating adiponectin concentrations and HOMA-IR was clearly established in the current study in healthy Korean subjects. In particular, the *G* allele at SNP 276 was associated with lower plasma adiponectin and higher IR in relatively obese persons but not in lean persons. We showed that, independent of other measured environmental factors, carriers of the *G* allele at position 276 might be at high CVD risk because of higher fasting triacylglycerol, small dense LDL concentrations, oxidative stress, and smaller LDL particle size. Therefore, we could suggest that this genetic marker may help in the identification of subjects who are at greater risk of CVD risk, so that preventive programs to reduce CVD in later life could be specifically targeted at these subjects.

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All of the authors were involved in the development of the study protocol and the experimental design. The recruitment and schedule of the subjects were managed by YJ and JSC. Sample collection and experiments were performed by JSC, SJK, JYK, and HC. DNA analysis was performed by JEL. Data were analyzed by OYK and JMO. JHL wrote the draft manuscript with contribution from JMO and YJ. All the authors contributed to, read, and commented on the submitted and revised manuscripts. None of the authors had any personal or financial conflict of interest.

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